

CLAIMS

What is claimed is:

- 5 ~~Sub A1~~
1. A modified serine hydrolase that catalyzes a transamidation or a transpeptidation or a transesterification reaction, said protease having one or more amino acid residues in a subsite replaced with a cysteine, wherein the cysteine is modified by replacing the thiol hydrogen in the cysteine with a substituent group providing a thiol side chain comprising a moiety selected from the group consisting of a polar aromatic substituent, an alkyl amino group with a positive charge, a chiral substituent, a heterocyclic substituent, and a glycoside.
 - 10 2. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transamidation.
 3. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transpeptidation.
 - 15 4. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transesterification.
 5. The modified serine hydrolase of claim 1, wherein said serine hydrolase is selected from the group consisting of an alpha/beta serine hydrolase, a subtilisin type serine protease, and a chymotrypsin serine protease.
 - 20 6. The modified serine hydrolase of claim 1, wherein said serine hydrolase is a subtilisin.
 7. The modified serine hydrolase of claim 6, wherein said serine hydrolase catalyzes a transamidation and is stereoselective.
 8. The modified serine hydrolase of claim 6, wherein the amino acid replaced with a cysteine is an amino acid in the S₁, S₁', or S₂ subsite.

9. The modified serine hydrolase of claim 8, wherein the amino acid replaced with a cysteine is selected from the group consisting of asparagine, leucine, methionine, and serine.

5 ~~8/10~~ 10. The modified serine hydrolase of claim 8, wherein said amino acid is selected from the group consisting of amino acid 156 in the S₁ subsite, amino acid 166 in the S₁ subsite, amino acid 217 in the S₁' subsite, amino acid 222 in S₁' subsite and amino acid 62 in the S₂ subsite.

10 11. The modified serine hydrolase of claim 1, wherein said substituent is selected from the group consisting of an oxazolidinone, a C₁ to C₁₅ alkyl amino group with a positive charge, and a glycoside.

12. The modified serine hydrolase of claim 11, wherein said glycoside is selected from the group consisting of a monosaccharide, a disaccharides, and an oligosaccharide comprising pentoses and hexoses.

15 ~~8/10~~ 13. The modified serine hydrolase of claim 1, wherein said substituent is selected from the group consisting of the substituents listed in Figure 2.

20 14. The modified serine hydrolase of claim 1, wherein said substituent is selected from the group consisting of (R)-2-methoxy-2-phenyl-ethyl-thiol, (S)-2-methoxy-2-phenyl-ethyl-thiol, (R)-2-hydroxy-2-phenyl-ethyl-thiol, (S)-2-hydroxy-2-phenyl-ethyl-thiol, N-(3'-thio-propyl)-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-benzyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(R)-4-phenyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-phenyl-2-oxazolidinone, N-(2'-thioethyl)-(R)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-benzyl-2-oxazolidinone, N-(3'-thio)-(3aR-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one, and N-(3'-thio)-(3aS-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one.

25 15. A chemically modified mutant subtilisin, said subtilisin having one or more amino acid residues selected from the S₁, S₁', or S₂ subsites replaced with a cysteine, wherein the cysteine is modified by replacing the thiol hydrogen in the cysteine with a substituent group providing a thiol side chain comprising a moiety selected from the group

consisting of a polar aromatic substituent, an alkyl amino group with a positive charge, an alkyl group bearing a negatively charged moiety, and a glycoside.

16. The subtilisin of claim 15, wherein the amino acid residue replaced with a cysteine is selected from the group consisting of amino acid 62, amino acid 156, amino acid 166, amino acid 217, and amino acid 222.

17. The subtilisin of claim 16, wherein said substituent is selected from the group consisting of an oxazolidinone, a C₁ to C₁₅ alkyl amino group with a positive charge, a C₁ to C₁₅-SO₃⁻, C₁ to C₁₅-CO₂⁻, and a glycoside.

18. The subtilisin of claim 17, wherein said glycoside is selected from the group consisting of a monosaccharide, a disaccharides, an oligosaccharide comprising pentoses and hexoses.

19. The subtilisin of claim 16, wherein said substituent is selected from the group consisting of the substituents listed in Figure 2.

20. The subtilisin of claim 16, wherein said substituent is selected from the group consisting of (R)-2-methoxy-2-phenyl-ethyl-thiol, (S)-2-methoxy-2-phenyl-ethyl-thiol, (R)-2-hydroxy-2-phenyl-ethyl-thiol, (S)-2-hydroxy-2-phenyl-ethyl-thiol, N-(3'-thio-propyl)-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-benzyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(R)-4-phenyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-phenyl-2-oxazolidinone, N-(2'-thioethyl)-(R)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-benzyl-2-oxazolidinone, N-(3'-thio)-(3aR-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one, and N-(3'-thio)-(3aS-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one.

21. A method of forming a peptide bond, said method comprising contacting a compound comprising an ester substrate with a serine hydrolase of claim 1 or 15 and a primary amine under conditions whereby said hydrolase catalyzes the formation of a peptide bond.

22. The method of claim 21, wherein said compound comprising an ester substrate is an acyl donor and said primary amine is an acyl acceptor.

23. The method of claim 22, wherein said acyl acceptor is an amino acid amide.
24. The method of claim 23, wherein said amino acid amide is present in a peptide.
- 5 25. The method of claim 22, wherein said acyl acceptor is an L-amino acid amide.
26. The method of claim 22, wherein said acyl acceptor is a D-amino acid amide.
- 10 27. The method of claim 22, wherein said ester substrate is an amino acid ester.
28. The method of claim 27, wherein said amino acid ester is present in a peptide.
29. The method of claim 22, wherein said ester substrate is an L-amino acid ester.
- 15 30. The method of claim 22, wherein said ester substrate is a D-amino acid ester.
31. A method of resolving racemic primary and secondary alcohols using a transesterification reaction, said method comprising contacting said racemic primary or secondary alcohols with a serine hydrolase of claims 1 or 15 and an acyl donor whereby said
20 serine hydrolase catalyzes a transesterification reaction resolving said racemic primary or secondary alcohol.
32. The method of claim 31, wherein said primary or secondary alcohol is selected from the group consisting of an aliphatic alcohol, an aromatic alcohol, and a heterocyclic alcohol.
- 25 33. The method of claim 31, wherein said primary or secondary alcohol is selected from the group consisting of 2-phenyl-1-propanol, 2-methyl-1-pentanol, and 2 octanol.

34. The method of claim 31, wherein said acyl donors are selected from the group consisting of carboxylic acid esters and activated esters.

35. The method of claim 34, wherein said carboxylic acid esters are selected from the group consisting of alkyl carboxylic esters, and aralkyl esters.

5 36. The method of claim 34, wherein said activated ester is selected from the group consisting of a monohaloalkyl, a dihaloalkyl, and a trihaloalkyl.

37. The method of claim 31, wherein said modified mutant enzyme is selected from the group consisting of L217C-(CH₂)₂-SO₃⁻, N62C-(CH₂)₂-SO₃⁻, and N62C-S-CH₃.

10 38. A method of attaching a chiral moiety to a substrate via a transamidation, a transesterification, or a transpeptidation reaction, said method comprising contacting said substrate having a reactive site suitable for a transesterification or a transamidation, and said moiety with a catalytic serine hydrolase of claims 1 or 15 under conditions whereby said chiral moiety is covalently coupled to said substrate.

15 39. The method of claim 38, wherein said moiety is a chiral is selected from the group consisting of a D amino acid, an L-amino acid, an acyclic aliphatic, a cyclic aliphatic, an aralkyl R-carboxylic acid, and aralkyl S-carboxylic acid, an aromatic R-carboxylic acid, and an aromatic S-carboxylic acid.

20 40. The method of claim 39, wherein said reaction is preferential for a moiety of one chirality.

41. The method of claim 39, wherein said transesterification results in an enantiomerically biased product.

42. The method of claim 38, wherein said substrate is an amino acid or a polypeptide.

25 43. A method of incorporating an amino acid into a polypeptide, said method comprising contacting an amino acid ester with a catalytic serine protease of claim 1 or 15 and an amino acid primary amine under conditions whereby said serine hydrolase

catalyzes the formation of a peptide bond between the amino acid of said amino acid ester and the amino acid of the amino acid amine.

44. The method of claim ~~43~~, wherein said amino acid ester is an acyl donor and said amino acid amine is an acyl acceptor.

5 45. The method of claim ~~43~~, wherein said amino acid amide is present in a peptide.

46. The method of claim ~~45~~, wherein said amino acid amide is an L-amino acid amide.

10 47. The method of claim ~~45~~, wherein said amino acid amide is a D-amino acid amide.

48. The method of claim ~~43~~, wherein said amino acid ester is an L-amino acid ester.

49. The method of claim ~~43~~, wherein said amino acid ester is a D-amino acid ester.

15 50. The method of claim ~~43~~, wherein said amino acid ester is present in a peptide.

~~51. A method of producing a chemically modified mutated serine hydrolase, said method comprising providing a serine hydrolase wherein one or more amino acids have been replaced with cysteine residues; and replacing the thiol hydrogens in the cysteine residues with a substituent group providing a thiol side chain comprising a moiety selected from the group consisting of consisting of a polar aromatic substituent, an alkyl amino group with a positive charge, and a glycoside.~~

25 52. The method of claim 51, wherein said hydrolase is selected from the group consisting of an alpha/beta serine protease, a subtilisin type serine protease, and a chymotrypsin serine protease.

53. The method of claim ~~51~~, wherein said hydrolase is a subtilisin.

54. The method of claim ~~53~~, wherein the amino acid replaced with a cysteine is an amino acid in the S₁, S₁', or S₂ subsite.

55. The method of claim ~~53~~, wherein the amino acid replaced with a cysteine is selected from the group consisting of asparagine, leucine, methionine, and serine.

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56. The method of claim ~~53~~, wherein said amino acid is selected from the group consisting of amino acid 156 in the S₁ subsite, amino acid 166 in the S₁ subsite, amino acid 217 in the S₁' subsite, amino acid 222 in S₁' subsite and amino acid 62 in the S₂ subsite.

57. The method of claim ~~53~~, wherein said substituent is selected from the group consisting of an oxazolidinone, a C₁ to C₁₅ alkyl amino group with a positive charge, and a glycoside.

58. The method of claim ~~57~~, wherein said glycoside is selected from the group consisting of a monosaccharide, a disaccharides, and an oligosaccharide comprising pentoses and hexoses.

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59. The method of claim ~~53~~, wherein said substituent is selected from the group consisting of the substituents listed in Figure 2.

60. The method of claim ~~53~~, wherein said substituent is selected from the group consisting of (R)-2-methoxy-2-phenyl-ethyl-thiol, (S)-2-methoxy-2-phenyl-ethyl-thiol, (R)-2-hydroxy-2-phenyl-ethyl-thiol, (S)-2-hydroxy-2-phenyl-ethyl-thiol, N-(3'-thio-propyl)-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-benzyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(R)-4-phenyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-phenyl-2-oxazolidinone, N-(2'-thioethyl)-(R)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-benzyl-2-oxazolidinone, N-(3'-thio)-(3aR-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one, and N-(3'-thio)-(3aS-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one.

61. The method of claim ~~53~~, wherein said method further comprises screening the modified serine hydrolase for an activity selected from the group consisting of a transesterification activity, a transamidation activity, and a transpeptidation activity.

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62. The method of claim 61, wherein said activity is stereoselective.

62. The method of claim 61, wherein said activity is stereoselective.